1982

Effectiveness of Some Irrigants in the Elimination of Bacteroides Melaninogenicus from the Root Canal System: An In Vitro Study

David Beacom Foley

Loyola University Chicago

EFFECTIVENESS OF SOME IRRIGANTS IN THE ELIMINATION OF *BACTEROIDES MELANINOGENICUS* FROM THE ROOT CANAL SYSTEM: AN IN VITRO STUDY

by

David Beacom Foley

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of

Master of Science

May

1982

ACKNOWLEDGEMENTS

I wish to thank Dr. Franklin S. Weine for his guidance in my life, both personally and professionally. Thank you to Dr. James Hagen and Dr. Scott Shellhammer for their time and contribution to this thesis.

I especially wish to thank Dr. Alan Azar and Dr. Brent Sonnenberg for their friendship and encouragement, for without them I would not have been able to attain this goal.

DEDICATION

To my wife, Murph, whose love makes my work worthwhile, and to my parents, whose support and love have led me to achieve this goal.

VITA

The author, David Beacom Foley, is the son of Dan Beacom Foley and Cynthia (Johnson) Foley. He was born June 8, 1952, in Denver, Colorado.

His elementary education was obtained in the public schools of Denver, Colorado, and secondary education at the South High School, Denver, Colorado, where he was graduated in June, 1970.

In September, 1970, he entered the Colorado State University, Fort Collins, Colorado.

In September, 1972, he entered Regis College, Denver, Colorado, where he was graduated with honors in August, 1975, with the degree of Bachelor of Science with a major in chemistry.

In June, 1975, he entered the University of Colorado School of Dentistry, where he received the degree of Doctor of Dental Surgery in May, 1979.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Many endodontic problems, including pain, foul odor, and sinus tract formation, have been recently attributed to the anaerobic bacterium Bacteroides melaninogenicus. On the basis of these findings and realizing that B. melaninogenicus may be a significant endodontic pathogen, the rapid elimination of the organism from the root canal system would seem to be desirable.

Cleansing and shaping of the root canal provides for the removal of necrotic tissue and debris, as well as reducing the microbial population. As an adjunct to this debridement process irrigation solutions are used. One method of canal irrigation that is advocated involves the alternate use of Gly-Oxide, a commercial preparation of 10 percent carbamide (urea) peroxide in an anhydrous glycerol base, with sodium hypochlorite. When these two solutions are mixed, an effervescent release of oxygen occurs. It may be that irrigation with these agents and the resultant release of oxygen, in combination with their antimicrobial qualities, will be effective in killing the strictly anaerobic B. melaninogenicus.

It is the purpose of this study to determine the in vitro effectiveness of combined irrigation with sodium

hypochlorite and Gly-Oxide in the elimination of B. melaninogenicus from the root canal system, as compared to the more commonly used method utilizing only sodium hypochlorite.

REVIEW OF THE LITERATURE

Role of microorganisms in endodontic disease

Microorganisms have long been implicated as a cause of disease in the pulp and periapical tissues. However, whether bacteria actually cause or only contribute to these diseases is not clear. Ever since 1894 when W.D. Miller (1) first identified bacteria in the pulpal tissues, numerous investigators have attributed pulp disease to the presence of microorganisms. No specific studies reporting the mechanism of bacterial action have been published, although papers correlating endodontic success or failure with culture results have been presented (2-6). Since most of these studies report greater endodontic success after negative culture, it was assumed that bacteria adversely affect endodontic results.

In 1965, Kakehashi and others (7) were able to demonstrate that bacteria could cause pulpal inflammation and destruction. Pulps were exposed in both conventional and germ-free laboratory rats, and left open to the environment. Pulp necrosis and abscesses occurred in the infected rats, while healing and repair was seen in the germ-free rats.

Torneck (8) reviewed the role of microorganisms and

offered the following hypotheses:

1. The actions of microorganisms are among the causes in the prevention or delay of healing. However, bacteria are only effective when other irritating factors such as periodontal disease or overinstrumentation have already initiated an inflammatory response.

2. Not all microorganisms can influence periapical repair. The type and number of bacteria are significant in each case.

Bartels and others (9) attempted to determine the causative factors of "flare-ups" during endodontic therapy. A "flare-up" was defined as a condition of pain or swelling that required an emergency visit. Among other things, they concluded that no relationship exists between endodontic symptoms and any specific bacterial isolates.

Not until 1976 were any correlations shown between a specific root canal microorganism and any endodontic disease or clinical manifestation. In that year, Sundqvist (10) undertook an involved bacteriologic study of necrotic pulps associated with periapical lesions, and isolated B. melaninogenicus, plus at least one other anaerobe, from every tooth that had pain involvement. B. melaninogenicus was never

isolated from a pain-free tooth. He concluded that acute periapical inflammation is induced by specific combinations of B. melaninogenicus with other anaerobic bacteria (Peptostreptococcus anaerobius, Peptostreptococcus micros, or Campylobacter sputorum). He further stated that B. melaninogenicus is always necessary for this condition. In a follow-up study, Griffee and others (11) found a significant relationship between B. melaninogenicus and pain, sinus tract formation, and foul odor. Other relationships were suggested between the organism and the presence of apical sensitivity, and local swelling. To date, B. melaninogenicus is the only microorganism that has been identified as being directly associated with endodontic symptoms.

Significance of anaerobic bacteria in endodontics

Until the early 1970s, most investigators agreed that the predominate microbial species infecting the root canal were members of the facultative anaerobic alpha-hemolytic streptococci (12-19). These gram-positive organisms included such species as Streptococcus mitis, Streptococcus salivarius, Streptococcus mutans, and Streptococcus sanguis. Other frequently isolated aerobic or facultative organisms were Streptococcus faecalis, Staphlococcus epidermidis, Corynebacterium, lactobacilli, and micrococci. These bacteria were isolated using standard aerobic culture

techniques and media which included brain-heart infusion broth, thioglycolate broth, and trypticase-soy broth.

Recognizing that the root canal system was probably also colonized by some strict anaerobic organisms, Leavitt, Naidorf, and Shugaevsky (20) introduced agar into the media (e.g., trypticase-soy broth with 0.1 percent agar). This so-called deep broth technique helped in the detection of some anaerobes such as Veillonella, Bacteroides, Fusobacteria, and Campylobacter species. Nonetheless, many obligate anaerobes remained undetected.

Early attempts to isolate anaerobic bacteria met with varying degrees of success. By using various unsophisticated anaerobic methods, anaerobes were found to comprise from 15 percent (13) to 62 percent (17) of the total number of strains isolated. During these investigations, several interesting observations concerning anaerobic bacteria were made:

1. Leavitt, Naidorf, and Shugaevsky (15) thought that anaerobic bacteria might be responsible for some endodontic failures.

2. Engstrom and Frostell (18) observed that bacteria were always present in teeth with a "gangrenous odor". They felt that this odor

was probably due to anaerobes.

3. Möller (19) believed that the strictly anaerobic, non-sporulating bacteria might be of importance in the pathological changes of the periapical region. (B. melaninogenicus?)

Finegold (21) stated that with appropriate techniques, anaerobes would be found to be the most important causes of dental infection. Using strict anaerobic techniques, Fulghum, Wiggins, and Mullaney (22) concluded that anaerobic bacteria play a larger role in the necrotic pulp than was previously thought. They suggested further studies to determine the extent and role of anaerobic microorganisms in endodontic disease.

With the development of more sophisticated anaerobic methods, specifically the Virginia Polytechnic Institute (VPI) method using roll tubes and prereduced media, and the anaerobic glove box, several further investigations were performed (23-28). Results of these studies ranged from Bergenholtz (23), who isolated 78 percent anaerobic bacteria, to Wittgow and Sabiston (25), who were able to isolate 97 percent obligate anaerobic organisms. These findings suggest that anaerobes are quite prevalent in the necrotic dental pulp, and are probably the predominate microflora.

Bacteroides melaninogenicus

Oliver and Wherry (29) first described B. melaninogenicus on blood agar plates as a black, dry growth around which hemoglobin disappears. Microscopically the organism is seen as gram-negative, non-acid fast, non-motile, polymorphous rods. The black pigment was initially thought to be melanin, but was later shown to be hematin (30).

Williams and others (31) described three subspecies of B. melaninogenicus. They are B. melaninogenicus ss. melaninogenicus, B. melaninogenicus ss. intermedius, and B. melaninogenicus ss. asaccharolyticus. The subspecies differ from one another in cell wall composition, DNA base composition, and physiologic tests. The authors indicated that substantial differences exist between the subspecies, and that it may be inappropriate for them to be retained in the same species. In 1977, Bacteroides asaccharolyticus was established as a species distinct from B. melaninogenicus because of metabolic, serological, and genetic differences (32). Further differences between oral and nonoral strains of B. asaccharolyticus have been demonstrated, and the separate species Bacteroides gingivalis has been suggested (33).

B. melaninogenicus grows preferentially with other bacteria, and exhibits slow and meager growth in pure culture (34). Trying different experimental approaches,

Socransky and Gibbons (35) confirmed the essential role of B. melaninogenicus in mixed anaerobic infections. They observed that B. melaninogenicus by itself caused little or no transmissible infection, but in combination with other bacteria, infection could be experimentally induced. In an analysis of one mixed infection, various recombinations of pure cultures from which B. melaninogenicus was omitted always failed to produce infection. Other strains of Bacteroides could be omitted and infection would still occur.

When isolated from infections, B. melaninogenicus is always found in association with other microorganisms. Two ideas have been proposed to explain this finding:

1. The other organisms provide B. melaninogenicus with vitamin K, which it requires for growth (36,37). Gibbons and MacDonald (37) stated that B. melaninogenicus requires a "growth factor" which is provided by other organisms. This "growth factor" could be replaced by menadione or other compounds of the vitamin K group.

2. The pathogenicity of B. melaninogenicus is dependent upon the production of collagenase, which in turn is dependent upon the presence

of other bacteria. MacDonald, Socransky, and Gibbons (38) felt that the pathogenicity was enhanced because the other microorganisms use an end product of collagenase activity to encourage the production of more collagenase by removing a feedback repressor from the environment.

The pathogenicity of B. melaninogenicus is probably due to a combination of the production of tissue destructive enzymes, hyaluronidase, chondroitin sulfatase, gelatinase, and collagenase, and the release of endotoxin (39). Studies by MacDonald, Gibbons, and Sawyer (40,41) have shown that B. melaninogenicus reduces hydroxyproline, and is therefore capable of hydrolyzing native collagen. Schein and Schilder (42) found that pulps in non-vital teeth contained more endotoxin than those which were asymptomatic. When it is considered that B. melaninogenicus releases endotoxin and destructive enzymes, these results seem to reinforce Sundqvist's (10) findings that all symptomatic teeth were positive for B. melaninogenicus.

Oxygen tolerance

Molecular oxygen in itself is not cytotoxic, but when reacted with the anaerobic cell, toxic oxygen products,

including hydrogen peroxide and the superoxide radical, are generated (43). It is these toxic products that are responsible for the destruction of the cell wall, and lysis of the cell.

A common misconception is that obligate anaerobic bacteria will be killed in the presence of oxygen, but anaerobes possess varying degrees of oxygen tolerance. Oxygen tolerance seems to be determined by superoxide dismutase (SOD) activity and oxygen reduction rates (44). The cellular presence of SOD, and possibly catalase and peroxidase, imparts an aerotolerance to the bacteria. Rolfe and others (44) were unable to detect any SOD, catalase, or peroxidase activity, and found low levels of oxygen reduction in B. melaninogenicus. They classified the organism as being oxygen intolerant. Other investigators have been able to detect low levels of SOD (45) and catalase (46) activities in some strains of B. melaninogenicus, suggesting some ability to tolerate oxygen.

Oxygen tolerance is also a function of dissolved oxygen concentration and duration of exposure. Loesch (47) demonstrated that B. melaninogenicus exhibited variable growth in 8 percent oxygen and no growth in 10 percent oxygen.

Irrigation of the root canal

Irrigation is a significant phase of the chemomechanical debridement of the root canal. The irrigant helps in the physical removal of remaining pulp and dentin fragments, acts as a lubricant to facilitate canal preparation, and aids in reducing the microbial population. Historically, various agents have been advocated for use as endodontic irrigants. Prader (48) recommended a stream of hot water, while Blechman and Cohen (49) suggested a 30 percent solution of urea. Walker (50) advised the use of sodium hypochlorite, while Coolidge and Kessel (51) advocated a solution of chloramine. Carbamide peroxide in glycerine (Gly-Oxide) was proposed by Stewart and others (52). Nichols (53), and Grahnen and Krasse (54) evaluated other irrigating solutions (distilled water, saline, a quaternary ammonium compound, and a polyantibiotic). Grossman (55) preferred a combination of a reducing agent (5 percent sodium hypochlorite) and an oxidizing agent (3 percent hydrogen peroxide), used alternately. He claimed that the resulting release of nascent oxygen, combined with the antimicrobial properties of both solutions. helped to destroy as well as remove microorganisms from the root canal. Other chemical agents suggested for irrigation include sulfuric acid (56), sodium hydroxide (57), and papain (58).

Several investigations have been undertaken to evaluate the effect irrigation has on debris removal. Baker and others (59) utilized the scanning electron microscope and found that 70 percent more tags of pulp tissue and dentin fragments were removed when an irrigant was used. They concluded that debris removal was a function of quantity, not type, of irrigant. McComb and Smith (60) studied several irrigating solutions and showed sodium hypochlorite to be the most effective irrigant for removing loose debris. Vande Visse and Brilliant (61) observed root canals that had been instrumented with and without irrigation. Their findings indicated that dentin shavings remained in the canals when instrumentation was attempted without irrigation.

The depth of irrigant penetration is dependent upon the size of the root canal. Ram (62) enlarged canals to sizes 25, 40, and 60, and then filled them with a radiopaque substance. The canals were then irrigated. It was found that the radiopaque material was washed out of only the coronal half of the root canal when it was enlarged to size no. 25, while the entire canal was cleared when enlarged to a no. 40 or no. 60 instrument. Ram concluded that irrigation is more complete and effective in a properly enlarged canal.

As suggested by Grossman (55), and Stewart and others (52), the alternating use of an oxygenating solution

(hydrogen peroxide, or Gly-Oxide) with sodium hypochlorite is widely used, and has been the subject of several studies. Svec and Harrison (63) irrigated forty teeth, half of which were flushed alternately with 5.25 percent sodium hypochlorite and 3 percent hydrogen peroxide, while the other half were irrigated with normal saline. Microscopic examination, after sectioning the teeth, indicated that the alternating irrigation with sodium hypochlorite and hydrogen peroxide was significantly more effective in cleaning the canal system at 1 mm and 3 mm from the apex. No difference was observed at the 5 mm level. A recent study by the same authors (64) indicated that alternate irrigation with 5.25 percent sodium hypochlorite and 3 percent hyrdogen peroxide did not produce significantly cleaner canals than did 5.25 percent sodium hypochlorite alone. Marshall, Massler, and Dute (65) demonstrated that, when sodium hypochlorite and hydrogen peroxide are used alternately, a significant increase in dentin permeability results. The same solutions used separately produced markedly less permeability. This suggests that combined irrigation may be more effective in elimination of bacteria within the dentin. Using absorbent paper points contaminated with S. faecalis, Harrison and Hand (66) evaluated the antibacterial effect of a solution formed by 5.25 percent sodium hypochlorite and 3 percent hydrogen peroxide (not alternated). The results indicated

that the solution had no bactericidal effectiveness against the test organism. Brown and Doran (67) found no difference between the ability of 5 percent sodium hypochlorite, and the combination of 5 percent sodium hypochlorite and 3 percent hydrogen peroxide, to float dentin fragments from a simulated root canal.

Sodium hypochlorite

Chlorine liberating solutions have been in use in medicine and dentistry for many years. Dakin (68) reported the use of a 0.5 percent sodium hypochlorite solution for the irrigation of wounds incurred by soldiers in World War I. Austin and Taylor (69,70) studied the necrotic tissue solvent action of Dakin's solution *in vitro* and *in vivo*. In 1936, Walker (50) introduced the use of a chlorinated soda solution to be used as an organic tissue solvent during root canal therapy. The use of Clorox as a source of 5.25 percent sodium hypochlorite was introduced by Lewis (71) in 1954.

One of the primary reasons that sodium hypochlorite has become the irrigant of choice in endodontic treatment is because of its ability to dissolve necrotic tissue. Grossman and Meiman (72) removed the pulps from freshly extracted teeth, and placed the tissue in various solutions. Sodium hypochlorite dissolved the pulps within 1 to 24

hours, and proved to be superior to such caustic solutions as 50 percent sulfuric acid, 25 percent sodium hydroxide, and 30 percent hydrochloric acid. Senia and others (73) irrigated the mesial root canals of mandibular molars with 5.25 percent sodium hypochlorite. The solvent action was evaluated by cross sectioning the teeth and exposing photomicrographs. The results indicated that full-strength Clorox was effective at removing tissue in the larger diameters of the root canal, but in the apical 3 mm its efficacy was questionable.

While sodium hypochlorite solution is used primarily for its tissue solvent properties, it also has powerful antimicrobial qualites (74). This bactericidal effect has been shown by Bence (75), Costigan (76), Cvek (77), and others. Shih and others (78) used extracted teeth to test the bactericidal effectiveness of sodium hypochlorite. The teeth were instrumented and then inoculated with the test organisms, S. faecalis and Staphylococcus aureas. Both bacteria were adequately eliminated by the sodium hypochlorite. In a recent study, Raphael and others (79) evaluated the effect that varying the temperature of sodium hypochlorite had on its antimicrobial qualities. No direct effect could be determined.

Spangberg (80) recommended diluting 5.25 percent

sodium hypochlorite to 0.5 percent because of its potential for toxicity. This recommendation was based upon the results of an in vitro cytotoxicity study in which suspensions of HeLa and L cells were subjected to prolonged contact with various concentrations of sodium hypochlorite. A 2.5 percent solution of sodium hypochlorite was shown by Trepagnier and others (81) to be equal to 5.25 percent sodium hypochlorite in its tissue dissolving properties. Harrison and others (66,82,83), in a series of articles, have shown that dilution of full-strength Clorox significantly decreases the necrotic tissue dissolution property, as well as the antimicrobial effect. In a clinical investigation relating painful episodes with the use of saline, full-strength sodium hypochlorite, and sodium hypochlorite alternated with hydrogen peroxide, no difference in the incidence of interappointment pain resulting from the use of these irrigants was reported (82).

The mechanism by which sodium hypochlorite exerts its solvent and antibacterial action has not been studied. Some clue to its method of action may come from an analysis of the lysosomal granules and the myeloperoxidase system. Hypochlorite ions are believed to chlorinate the cell wall, and lead to the degradation of the amino acid content of the wall (84). The sodium hypochlorite that is used as an endodontic irrigant may act in a similar manner.

Gly-Oxide - glycerite of carbamide peroxide

Oxygenating agents, particularly hydrogen peroxide, have been used in the healing arts because of their antimicrobial properties, and because they debrided necrotic tissue in such a discernable manner - debris is literally bubbled away. Unfortunately, hydrogen peroxide has several disadvantages: it is unstable, has a short duration of action, and has undesirable topical effects upon extended usage.

In an effort to overcome the disadvantages, and retain the desirable properties of hydrogen peroxide, several compounds were investigated. One of these compounds was carbamide peroxide. Carbamide peroxide, when in contact with tissue catalase and/or peroxidase, catalyzes into carbamide and hydrogen peroxide (85). The resultant hydrogen peroxide then liberates water and oxygen in the presence of the same enzymes. This formulation then offers the necessary release of oxygen, possesses the ability (of the carbamide) to solubilize organic debris (86), provides the desired antimicrobial action, and aids in mechanical removal of debris by foaming.

Brown (87) recognized the potential usefulness of carbamide peroxide as an antibacterial solution and found that when combined with glycerol the compound became more

stable. Because of the viscosity of the glycerol, the duration of oxygen and carbamide action was also increased. Follow-up studies confirmed the bactericidal and bacteriostatic properties of the solution (88-93). Brown and others (88) compared the action of carbamide peroxide in glycerol on anaerobic bacteria with that of 32 commercial antiseptic solutions. They found that with the exception of iodine, which was essentially equal in potency, the glycerite of carbamide peroxide was far more effective in bactericidal action than any of the other solutions. Thurmon and Brown (89) reported on treatment of patients with bacterial infection of the skin and mucous membranes, and suggested that glycerite of carbamide peroxide was superior to sulfonamides and other routine methods for the control of infections. In a comparison of glycerite of carbamide peroxide with several mercurial solutions, Brown and others (90) showed that glycerite of carbamide peroxide was most effective against gram-positive organisms, while the mercurials were most effective on gram-negative bacteria. Slantez and Brown (91,92) were able to demonstrate an appreciable reduction in the number of oral microorganisms when glycerite of carbamide peroxide was used as a mouth rinse.

Carbamide peroxide with glycerol has been shown to be essentially non-allergenic, non-irritating, and non-toxic (93). After studying the tissue tolerance of 4 percent

carbamide peroxide in anhydrous glycerol, Brown (94) concluded that, for the general population, the chance for an allergic reaction would be less than 1 percent. Gillan (95) investigated the apical tissue effects resulting from root canal irrigation with Gly-Oxide. He concluded that Gly-Oxide was no more irritating to the periapical tissues than was normal saline.

Gly-Oxide has been used in several phases of dentistry as an aid for debridement and to promote healing. Cobe and others (96) advocated the use of Gly-Oxide in the treatment of acute necrotizing gingivitis (ANUG), as a debriding agent to aid in hygiene, and to stimulate healing following scaling and curettage. Epstein (97) showed that it is an aid in controlling the severity of gingival inflammation. It was tested for its ability to initiate early healing of extraction wounds, and as an aid to the healing of dry sockets, by Wagner (98). Healing was found to be accelerated and enhanced by the Gly-Oxide, as compared to placebo and saline rinses. Stewart (52) introduced Gly-Oxide as an endodontic irrigant. As advantages for the use of this solution, he listed that it acts as a lubricant for canal preparation and has a prolonged action which allows time to work the material down into the canals.

MATERIALS AND METHODS

This study was designed on the premise that B. melaninogenicus is a significant endodontic pathogen, and that its prompt elimination from the root canal system is desirable. Recognizing the inadequacies of working in an in vitro environment, a considerable effort was made to mimic the clinical situation as closely as possible. The purpose of this research was to determine whether combined agent (sodium hypochlorite and glycerite of carbamide peroxide) root canal irrigation offers any advantage over single agent (sodium hypochlorite or glycerite of carbamide peroxide alone) irrigation, in the eradication of B. melaninogenicus.

Bacteriologic media

Two types of media were used during this project. Plates of enriched Todd-Hewitt agar with 5 percent sheep blood, and friction-cap tubes of enriched Todd-Hewitt broth were prepared. The plates contained: agar*, Todd-Hewitt broth**, and 5 percent defibrinated sheep blood***, enriched with 0.5 percent yeast extract**, 0.05 percent hemin**, and 0.0005 percent menadione**. The tubes contained the same

---

* Difco Laboratories, Detroit, MI
** Baltimore Biological Laboratories, BBL, Cockeysville, MD
*** Ovine Laboratories, Chicago, IL

components as the plates, excluding the agar and blood.

Microorganisms

The anaerobic microorganisms used throughout this investigation were Bacteroides melaninogenicus ss. intermedius and Peptostreptococcus anaerobius. B. melaninogenicus was a standard reference strain*. P. anaerobius was a clinical isolate obtained from the Foster G. McGaw Hospital, Maywood, IL.

In order to provide control over the number of organisms inoculated, the B. melaninogenicus and the P. anaerobius were kept in separate cultures, and then combined at the time of inoculation. The organisms were maintained on enriched Todd-Hewitt agar with 5 percent sheep blood. To prepare the inoculum, two typical colonies were transferred from blood plates to broth media. At 48 hours, fresh broth media was inoculated. After 24 hours of incubation, the cells were centrifuged and washed twice with sterile saline. Both the B. melaninogenicus and the P. anaerobius were then resuspended in fresh Todd-Hewitt broth to a concentration of 2×10^7 colony forming units/ml. The organisms were used at these concentrations for the test tube experiments. For inoculation into the teeth, the two adjusted cultures were

---

* ATCC 25261, American Type Culture Collection, Rockville, MD

combined in a 1:1 ratio immediately prior to their use.

Anaerobic conditions

Anaerobic conditions were established throughout the investigation by using a vented anaerobic jar*, which was evacuated and flushed with an anaerobic gas mixture**. To ensure that the jar remained anaerobic, an anaerobic indicator* was sealed in the jar.

Sterility and viability controls

Periodically throughout the study, Gram stains were made and blood agar streaked to verify viability of the organisms, continuation of the original pure cultures, and absence of contamination.

EXPERIMENT I

This portion of the study was designed to establish the sensitivity of the experimental organisms to varying concentrations of Clorox*** and Gly-Oxide**** using tube

---

* Baltimore Biological Laboratories, BBL, Cockeysville, MD
** 5 percent CO_2:10 percent H_2:bal. N_2, AnO_2 grade, Benster Specialty Gas Co., Franklin Park, IL
*** Contains 5.25 percent sodium hypochlorite; 4.00 percent sodium chloride; 0.20 percent sodium carbonate; and 0.005-0.015 percent free sodium hydroxide, Clorox Co., Oakland, CA
**** 10 percent carbamide peroxide in anhydrous glycerol, Marion Laboratories Inc., Kansas City, MO

dilutions.

A series of dilutions of Clorox and Gly-Oxide were prepared with sterile water. Ten milliliters of each solution (full-strength Clorox, Clorox diluted with sterile water 1:1,000, 1:5,000, 1:10,000, 1:15,000, full-strength Gly-Oxide, Gly-Oxide diluted with sterile water 1:1,000, 1:5,000, 1:10,000, 1:15,000, or sterile saline as a control) was aseptically dispensed into two sets of 11 tubes each. A 0.1 ml aliquot of B. melaninogenicus was added to one set of 11 tubes, and a 0.1 ml aliquot of P. anaerobius was added to the other set. All tubes were agitated during the experiment to ensure thorough mixing of the solutions and the bacteria. One-tenth of one milliliter samples were taken from each inoculated solution at 15, 30, and 45 seconds, 2 and 10 minutes, and 1 and 24 hours. Solutions remained in aerobic conditions throughout the sampling period. Samples were placed in 10 ml of enriched Todd-Hewitt broth and incubated anaerobically at 37^{o} C for 72 hours.

Turbidity of the culture media (observed against a black background) after the incubation period was used as the criterion of bacterial growth.

EXPERIMENT II

The purpose of this experiment was to evaluate the

bactericidal efficiency of Clorox and Gly-Oxide when used as root canal irrigants. An effort was made to duplicate the clinical situation as closely as was possible in an *in vitro* study.

Preparation of the teeth

One hundred maxillary central human incisor teeth were used in this study. The freshly extracted teeth were stored in normal saline solution until used experimentally. Using a no. 557 high-speed carbide bur*, the crowns of the teeth were removed, and a standard access opening was made. A no. 15 file was passed through the canal until it was seen to extend 1 mm beyond the apical foramen. This length was measured and was used as the working length for instrumentation. K-type files** were used to instrument the canals, and tap water served as the irrigant. A no. 15 file was used first, and each instrument size was used consecutively; the canals were prepared to the size of a no. 60 file used 1 mm through the apex. Additionally, the canals were flared (99) to three sizes beyond the no. 60 file. All instrumentation was performed employing only a filing motion in a circumferential manner. Each instrument was used for approximately 30 seconds.

---

* Premier Dental Products Co., Norristown, PA
** Star Dental Mfg. Co., Conshohocken, PA

Following instrumentation, the enlarged apices were sealed to prevent leakage. An epoxy resin* was mixed, painted on the apical 5 mm of each tooth, and allowed to dry for 1 hour. The teeth were then randomly separated into four groups of 25 and mounted in plaster to facilitate handling. Each resulting plaster block was labeled according to the irrigant to be used, and the respective teeth were numbered 1 through 25. The four blocks were wrapped separately in cloth towels, and autoclaved at 250° C for 1 hour. After cooling, the wrapped blocks were stored under anaerobic conditions until needed. In previous attempts which omitted this anaerobic storage, the bacteria did not survive inoculation into the teeth.

Inoculation of the teeth

The previously sterilized teeth were taken out of anaerobic storage for inoculation. A 0.01 ml inoculum, containing 10^5 cells each of *B. melaninogenicus* and *P. anaerobius* was introduced into the apical one-third of the root canal. A sterile 1 ml tuberculin syringe with a 27 gauge needle** was used. To prevent contamination, a sterile no. 3 cotton pellet*** was placed into the coronal access

---

* Carters General Purpose Epoxy, Carters Ink Co., Cambridge, MA
** Monoject, St. Louis, MO
*** Richmond Dental Cotton Co., Charlotte, NC

opening of each tooth. Todd-Hewitt broth was added to the cotton pellets to keep them moist and maintain a humid environment. In previous experiments without the cotton pellets, the media evaporated from the teeth during incubation, and the organisms did not survive. Aluminum foil was wrapped around each plaster block to further prevent contamination. The inoculated teeth were placed in the anaerobic jar with a beaker of sterile water to ensure a humid environment. The jar was flushed with an anaerobic gas mixture and incubated at 37° C for 48 hours. This incubation allowed the microorganisms to establish growth within the canals.

Irrigation procedures

After 48 hours of incubation, the teeth were removed from anaerobic conditions, and the irrigation procedures initiated. The four groups of 25 teeth each were irrigated as follows:

1. In group I each canal was irrigated with 12 ml of sterile 0.9% saline.
2. In group II each canal was irrigated with 10 ml of full-strength Clorox.
3. In group III each canal was irrigated with 10 ml of full-strength Gly-Oxide.
4. In group IV each canal was irrigated alternating 1 ml increments of full-strength

Gly-Oxide and full-strength Clorox until 5 ml of each solution had been delivered. Following irrigation of groups II, III, and IV, the canals were rinsed with 2 ml of sterile 0.9% saline to remove any remaining Clorox or Gly-Oxide.

Solutions were kept at room temperature and were delivered slowly over a period of 30 minutes for each canal. Gentle agitation with a no. 15 file was done during irrigation. Irrigation was performed with a 20 ml sterile disposable luerlock syringe and a 1½ inch 22 gauge needle*. Excess solutions were evacuated using a polyethylene tube connected to a high volume suction apparatus.

Effectiveness of the irrigation regimen

In order to evaluate the effectiveness of the various irrigation regimen, a modified wet paper point culture technique (100) was used. No attempt at quantification of the remaining viable organisms was made. The only interest was in the presence or absence of organisms within the root canal system.

A pre-irrigation culture was taken to establish that the organisms were initially viable. One sterile absorbent

---

* Monoject, St. Louis, MO

paper point* was used per tooth. The point was streaked on enriched Todd-Hewitt agar with 5 percent sheep blood. Following irrigation, each tooth was cultured using two sterile paper points. The first point was streaked on enriched Todd-Hewitt agar with 5 percent sheep blood; the second point was placed in 2 ml of enriched Todd-Hewitt broth, and was used to confirm the findings from the agar plate. The plates and tubes were incubated anaerobically at 37° C.

Observations for growth were made 5 days following the irrigation session. Bacterial growth was verified by turbidity of the tubes (observed against a black background), and differentiated by preparing Gram stains of characteristic colonies on the plates.

---

* Johnson and Johnson Dental Products Inc., New Brunswick, NJ

RESULTS

Experiment I · Tube dilutions

The results of exposure of B. melaninogenicus to various dilutions of Clorox over several time periods are shown in Table 1. B. melaninogenicus was quite effectively eliminated by all of the dilutions that were studied. Only at the 1:15,000 Clorox dilution did the bacteria survive past 15 seconds, and even at that dilution the viable organisms were killed by 30 seconds of exposure.

Results of exposing B. melaninogenicus to different concentrations of Gly-Oxide are reported in Table 2. The data indicate that B. melaninogenicus was eliminated by 15 seconds of contact with full-strength Gly-Oxide. At the 1:1,000, 1:5,000, and 1:10,000 Gly-Oxide dilutions B. melaninogenicus endured 1 minute of exposure, but was killed by 10 minutes. A 1:15,000 dilution of Gly-Oxide failed to kill B. melaninogenicus even after 24 hours. Similarly, a normal saline solution allowed B. melaninogenicus to survive for at least 24 hours.

The inhibition of P. anaerobius by Clorox dilutions appears in Table 3. At the 15 second sampling, P. anaerobius failed to grow after exposure to full-strength Clorox.

When diluted 1:1,000, Clorox suppressed P. anaerobius growth after 10 minutes but not after 1 minute of contact. P. anaerobius was not affected by Clorox diluted 1:5,000, 1:10,000, or 1:15,000 at the 1 hour sampling, but after 24 hours the organism failed to grow from all test dilutions of Clorox.

Table 4 shows the effect that Gly-Oxide dilutions had upon P. anaerobius. Full-strength Gly-Oxide killed P. anaerobius by 15 seconds of contact. P. anaerobius failed to grow after 1 minute of exposure to 1:1,000 Gly-Oxide, and after 10 minutes of exposure to 1:5,000 Gly-Oxide. Gly-Oxide dilutions of 1:10,000 and 1:15,000 eliminated the organism by the 1 hour sampling time. Saline had no effect upon P. anaerobius; the microorganism grew after 24 hours in a normal saline solution.

Experiment II · Irrigation studies

The data in Table 5 represents the findings before and after experimentally infected extracted teeth were irrigated with a saline solution. Prior to the irrigation, culturing confirmed that both B. melaninogenicus and P. anaerobius had established growth in all 25 teeth. After irrigation, the agar plates showed growth of both test organisms from all but one tooth; in that tooth neither organism was cultured. All 25 post-irrigation broth cultures were

positive for growth.

As shown in Table 6, all 25 teeth irrigated with full-strength Clorox were positive for B. melaninogenicus and P. anaerobius growth before the irrigation procedure. After the irrigation neither organism could be detected in the broth or on the agar plates.

Table 7 shows the findings when irrigation was accomplished using full-strength Gly-Oxide. Twenty-four of the 25 canals gave positive cultures for B. melaninogenicus and P. anaerobius prior to irrigation. One pre-irrigation culture was negative for both microorganisms. When streaked on agar ten of the 24 initial positive canals were positive for growth. Twelve of the broth cultures were positive. Of the positive cultures, three grew only P. anaerobius. No teeth were positive for B. melaninogenicus alone.

Results from combined irrigation with full-strength Clorox and full-strength Gly-Oxide appear in Table 8. Three teeth in this group had no growth prior to irrigation. Following irrigation, negative cultures on agar and in broth were obtained from all of the treated teeth.

In summary, 96 of 100 pre-irrigation cultures were positive for B. melaninogenicus and P. anaerobius growth. Irrigation with saline resulted in no negative cultures,

while irrigation with full-strength Clorox or full-strength Clorox with full-strength Gly-Oxide resulted in no positive cultures. A variable number of negative cultures (15 of 24 on agar, and 13 of 24 in broth) were obtained following irrigation with full-strength Gly-Oxide.

Table 1. Results of tube dilution studies of *Bacteroides melaninogenicus* inhibition by Clorox at various times.

	Time of exposure						
	15 sec	30 sec	45 sec	1 min	10 min	1 hr	24 hr
Clorox full strength	-	-	-	-	-	-	-
Clorox diluted 1:1,000	-	-	-	-	-	-	-
Clorox diluted 1:5,000	-	-	-	-	-	-	-
Clorox diluted 1:10,000	-	-	-	-	-	-	-
Clorox diluted 1:15,000	+	-	-	-	-	-	-
Saline (control)	+	+	+	+	+	+	+

- = No bacterial growth observed.

+ = Bacterial growth observed.

Table 2. Results of tube dilution studies of Bacteroides melaninogenicus inhibition by Gly-Oxide at various times.

	Time of exposure						
	15 sec	30 sec	45 sec	1 min	10 min	1 hr	24 hr
Gly-Oxide full strength	-	-	-	-	-	-	-
Gly-Oxide diluted 1:1,000	+	+	+	+	-	-	-
Gly-Oxide diluted 1:5,000	+	+	+	+	-	-	-
Gly-Oxide diluted 1:10,000	+	+	+	+	-	-	-
Gly-Oxide diluted 1:15,000	+	+	+	+	+	+	+
Saline (control)	+	+	+	+	+	+	+

- = No bacterial growth observed.

+ = Bacterial growth observed.

Table 3. Results of tube dilution studies of Peptostreptococcus anaerobius inhibition by Clorox at various times.

	Time of exposure						
	15 sec	30 sec	45 sec	1 min	10 min	1 hr	24 hr
Clorox full strength	-	-	-	-	-	-	-
Clorox diluted 1:1,000	+	+	+	+	-	-	-
Clorox diluted 1:5,000	+	+	+	+	+	+	-
Clorox diluted 1:10,000	+	+	+	+	+	+	-
Clorox diluted 1:15,000	+	+	+	+	+	+	-
Saline (control)	+	+	+	+	+	+	+

- = No bacterial growth observed.

+ = Bacterial growth observed.

Table 4. Results of tube dilution studies of *Peptostreptococcus anaerobius* inhibition by Gly-Oxide at various times.

	Time of exposure						
	15 sec	30 sec	45 sec	1 min	10 min	1 hr	24 hr
Gly-Oxide full strength	-	-	-	-	-	-	-
Gly-Oxide diluted 1:1,000	+	+	+	-	-	-	-
Gly-Oxide diluted 1:5,000	+	+	+	+	-	-	-
Gly-Oxide diluted 1:10,000	+	+	+	+	+	-	-
Gly-Oxide diluted 1:15,000	+	+	+	+	+	-	-
Saline (control)	+	+	+	+	+	+	+

- = No bacterial growth observed.

+ = Bacterial growth observed.

Table 5. Results of bacterial culturing of extracted teeth, inoculated with Bacteroides melaninogenicus and Peptostreptococcus anaerobius, before and after irrigation with saline.

	Pre-irrigation	Post-irrigation	
Tooth #	Plate[1]	Plate	Broth[2]
1	Bm[3],Pa[4]	Bm,Pa	+[5]
2	Bm,Pa	Bm,Pa	+
3	Bm,Pa	Bm,Pa	+
4	Bm,Pa	-[6]	+
5	Bm,Pa	Bm,Pa	+
6	Bm,Pa	Bm,Pa	+
7	Bm,Pa	Bm,Pa	+
8	Bm,Pa	Bm,Pa	+
9	Bm,Pa	Bm,Pa	+
10	Bm,Pa	Bm,Pa	+
11	Bm,Pa	Bm,Pa	+
12	Bm,Pa	Bm,Pa	+
13	Bm,Pa	Bm,Pa	+
14	Bm,Pa	Bm,Pa	+
15	Bm,Pa	Bm,Pa	+
16	Bm,Pa	Bm,Pa	+
17	Bm,Pa	Bm,Pa	+
18	Bm,Pa	Bm,Pa	+
19	Bm,Pa	Bm,Pa	+
20	Bm,Pa	Bm,Pa	+
21	Bm,Pa	Bm,Pa	+
22	Bm,Pa	Bm,Pa	+
23	Bm,Pa	Bm,Pa	+
24	Bm,Pa	Bm,Pa	+
25	Bm,Pa	Bm,Pa	+

1 Growth on enriched Todd-Hewitt agar.
2 Growth in enriched Todd-Hewitt broth.
3 B. melaninogenicus growth observed.
4 P. anaerobius growth observed.
5 Growth observed.
6 No growth observed.

Table 6. Results of bacterial culturing of extracted teeth, inoculated with Bacteroides melaninogenicus and Peptostreptococcus anaerobius, before and after irrigation with full-strength Clorox.

	Pre-irrigation	Post-irrigation	
Tooth #	Plate[1]	Plate	Broth[2]
1	Bm[3],Pa[4]	-[5]	-
2	Bm,Pa	-	-
3	Bm,Pa	-	-
4	Bm,Pa	-	-
5	Bm,Pa	-	-
6	Bm,Pa	-	-
7	Bm,Pa	-	-
8	Bm,Pa	-	-
9	Bm,Pa	-	-
10	Bm,Pa	-	-
11	Bm,Pa	-	-
12	Bm,Pa	-	-
13	Bm,Pa	-	-
14	Bm,Pa	-	-
15	Bm,Pa	-	-
16	Bm,Pa	-	-
17	Bm,Pa	-	-
18	Bm,Pa	-	-
19	Bm,Pa	-	-
20	Bm,Pa	-	-
21	Bm,Pa	-	-
22	Bm,Pa	-	-
23	Bm,Pa	-	-
24	Bm,Pa	-	-
25	Bm,Pa	-	-

1 Growth on enriched Todd-Hewitt agar.
2 Growth in enriched Todd-Hewitt broth.
3 B. melaninogenicus growth observed.
4 P. anaerobius growth observed.
5 No growth observed.

Table 7. Results of bacterial culturing of extracted teeth, inoculated with Bacteroides melaninogenicus and Peptostreptococcus anaerobius, before and after irrigation with full-strength Gly-Oxide.

	Pre-irrigation	Post-irrigation	
Tooth #	Plate[1]	Plate	Broth[2]
1	Bm[3],Pa[4]	-[5]	-
2	Bm,Pa	-	-
3	Bm,Pa	-	-
4	Bm,Pa	-	-
5	-	-	-
6	Bm,Pa	-	-
7	Bm,Pa	-	-
8	Bm,Pa	Bm,Pa	+[6]
9	Bm,Pa	-	-
10	Bm,Pa	Pa	+
11	Bm,Pa	Bm,Pa	+
12	Bm,Pa	-	-
13	Bm,Pa	-	+
14	Bm,Pa	Bm,Pa	+
15	Bm,Pa	-	-
16	Bm,Pa	Bm,Pa	+
17	Bm,Pa	Pa	+
18	Bm,Pa	Bm,Pa	+
19	Bm,Pa	-	+
20	Bm,Pa	-	-
21	Bm,Pa	Pa	+
22	Bm,Pa	-	-
23	Bm,Pa	Bm,Pa	+
24	Bm,Pa	Bm,Pa	+
25	Bm,Pa	-	-

1 Growth on enriched Todd-Hewitt agar.
2 Growth in enriched Todd-Hewitt broth.
3 B. melaninogenicus growth observed.
4 P. anaerobius growth observed.
5 No growth observed.
6 Growth observed.

Table 8. Results of bacterial culturing of extracted teeth, inoculated with Bacteroides melaninogenicus and Peptostreptococcus anaerobius, before and after irrigation with full-strength Clorox alternated with full-strength Gly-Oxide.

	Pre-irrigation	Post-irrigation	
Tooth #	Plate[1]	Plate	Broth[2]
1	Bm[3],Pa[4]	-[5]	-
2	Bm,Pa	-	-
3	Bm,Pa	-	-
4	Bm,Pa	-	-
5	-	-	-
6	Bm,Pa	-	-
7	Bm,Pa	-	-
8	Bm,Pa	-	-
9	Bm,Pa	-	-
10	Bm,Pa	-	-
11	-	-	-
12	Bm,Pa	-	-
13	Bm,Pa	-	-
14	Bm,Pa	-	-
15	Bm,Pa	-	-
16	Bm,Pa	-	-
17	Bm,Pa	-	-
18	Bm,Pa	-	-
19	-	-	-
20	Bm,Pa	-	-
21	Bm,Pa	-	-
22	Bm,Pa	-	-
23	Bm,Pa	-	-
24	Bm,Pa	-	-
25	Bm,Pa	-	-

1 Growth on enriched Todd-Hewitt agar.
2 Growth in enriched Todd-Hewitt agar.
3 B. melaninogenicus growth observed.
4 P. anaerobius growth observed.
5 No growth observed.

DISCUSSION

Bacteroides melaninogenicus was chosen for this study because of its suggested role as an endodontic pathogen. Analyzing the results of an investigation of teeth with necrotic pulps and periapical lesions, Sundqvist (10) found that B. melaninogenicus plus at least one other anaerobic bacterium were always isolated from teeth associated with episodes of pain. Furthermore, B. melaninogenicus was never cultured from a pain-free tooth. He concluded that a combination of B. melaninogenicus with other anaerobic microorganisms is capable of producing acute periapical inflammation. Additional evidence confirming the endodontic pathogenicity of B. melaninogenicus was given by Griffee and others (11). They were able to demonstrate relationships between the organism and pain, foul odor, sinus tract formation, apical sensitivity, and local swelling. Although a broad spectrum of microorganisms have been detected within the root canal, B. melaninogenicus is the only bacteria that has been identified as being causative for pulpal disease and its sequela.

The reported finding that B. melaninogenicus was always identified in conjunction with other organisms is important. Apparently the pathogenicity of B. melaninogenicus

is dependent upon other bacteria. Socransky and Gibbons (35) showed that after omitting B. melaninogenicus from numerous combinations of pure cultures, experimental infection could not be induced. Other species could be omitted, but if B. melaninogenicus were present infection would always occur. It is thought that pathogenicity of B. melaninogenicus is enhanced by the associated organisms providing vitamin K necessary for its growth, or by potentiating its collagenolytic action. Peptostreptococcus anaerobius is one anaerobic organism that is frequently isolated in conjunction with B. melaninogenicus. Because an effort was made to experimentally simulate the in vivo situation in vitro, it was decided that the combination of B. melaninogenicus and P. anaerobius would accurately mimic the clinical conditions.

Assuming B. melaninogenicus to be a significant endodontic pathogen, any methods which hasten its removal from the root canal system would seem to be of value for the prudent practitioner. No studies have been reported exploring the effects that present treatment modalities have upon the B. melaninogenicus population within the pulp canal space. Because of the scarcity of this potentially valuable information, this study was performed in an attempt to answer the following:

1. Are the commonly used root canal irrigants, sodium hypochlorite and Gly-Oxide,

bactericidal toward B. melaninogenicus? If so, how does solution concentration affect the killing property?

2. Is simple flushing of the canal with a non-active irrigant (saline) enough to eradicate B. melaninogenicus?
3. How effective is sodium hypochlorite irrigation in killing B. melaninogenicus within the root canal?
4. Does the addition of an oxygenating agent (Gly-Oxide) to the irrigation regimen offer any additional advantage over sodium hypochlorite alone?

Although this experiment was conducted in vitro, it is hoped that the answers to these questions are applicable in the in vivo milieu.

Significance of irrigant dilution

The data gathered from the tube dilution studies (Tables 1-4) indicate that both 5.25 percent sodium hypochlorite and full-strength Gly-Oxide effectively kill B. melaninogenicus and P. anaerobius within 15 seconds of contact. As the solutions are diluted, their germicidal abilities diminish, but still remain at clinically effective levels. When it is considered that most endodontic

appointments fall in the 30 to 60 minute time period, any solution resulting in negative growth after 10 minutes of exposure would probably be clinically efficacious, and even negative growth after 1 hour may be significant. Based upon this reasoning, it is apparent that both Gly-Oxide and 5.25 percent sodium hypochlorite diluted 1:1,000 would probably be effective in a clinical situation. Even Clorox diluted 1:15,000 might be considered effective against B. melaninogenicus.

Albeit some dilution of the solutions may not severely affect their bactericidal capabilities, one must ask if there is some logical reason for Gly-Oxide or Clorox being diluted. Spangberg (80) advocated diluting sodium hypochlorite to reduce the potential for periapical tissue irritation. As a result of a cytotoxicity study, he concluded that 5.25 percent sodium hypochlorite was too toxic for clinical use as an endodontic irrigant. This conclusion could not be substantiated in a recent clinical study reported by Harrison and others (82). Using interappointment pain as a measure of periapical inflammation, these investigators observed that the incidence and degree of pain associated with the use of 5.25 percent sodium hypochlorite (with or without 3 percent hydrogen peroxide) were actually less than those associated with the use of a normal saline solution. Similarly, Gillan (95) presented his findings on

the effects of Gly-Oxide irrigation upon the periapical tissues, and determined that full-strength Gly-Oxide was no more irritating than was normal saline. Other evidence suggests that dilution of 5.25 percent sodium hypochlorite adversely affects the necrotic tissue dissolution property (81,83), the debridement property (59,60,63), and the antibacterial property (66,78). Although this study suggests that lower concentrations of Clorox and Gly-Oxide are effective against B. melaninogenicus and P. anaerobius, other evidence shows that dilution is probably not necessary or advantageous.

Importance of control results

As the saline control groups for the irrigation studies (Tables 5-8) indicate, B. melaninogenicus or P. anaerobius were not eliminated by simple flushing of the canal. Virtually 100 percent of the cultures obtained following saline irrigation remained positive for both organisms.

The findings of this study do not support the recommendations of Baker and others (59). Based upon the results of scanning electron microscopic examination of root canals irrigated with various irrigating solutions, these investigators advocated the use of physiologic saline for irrigation purposes. They reported finding no statistical difference in the amount of microorganisms and debris removed

after irrigating with different solutions, and concluded that bacteria and debris removal was a function of quantity of irrigant, not nature of irrigant. When making this recommendation, Baker et al. failed to consider that the bacteria inhabiting the root canal are alive and capable of growth, and if not eliminated from or killed within the root canal they will continue to thrive. These investigators seem to have misinterpreted their results. Their study in conjunction with the present investigation indicates that bacteria cannot be eliminated by flushing the canal with a non-active solution. Because microorganisms cannot be mechanically washed from the root canal system, they must be killed within the canal, and the need for bactericidal irrigants such as sodium hypochlorite and Gly-Oxide is apparent.

In his textbook, Grossman (101) states that from a clinical standpoint, obligate anaerobes have little practical significance since such organisms are generally destroyed in the presence of air. Although this seems like a logical statement, it is not corroborated by the results of this research. The control teeth (saline irrigation) were open and exposed to air for at least 1 hour, approximately the length of an endodontic appointment, yet both B. melaninogenicus and P. anaerobius, each obligate anaerobes, continued to live. What Grossman failed to realize is that anaerobic bacteria possess the ability to tolerate exposure to

oxygen as a function of the enxymes superoxide dismutase, peroxidase, and catalase (44).

Efficacy of sodium hypochlorite irrigation

Considering the results of the simulated clinical irrigation studies (Tables 5-8), it becomes apparent that the commonly used sodium hypochlorite irrigation is extremely efficacious in disinfecting canals contaminated with B. melaninogenicus and P. anaerobius. Of the 25 artificially infected canals that were subjected to 5.25 percent sodium hypochlorite irrigation, not one resulted in a positive culture (Table 6).

The clinical relevance of these results is significant. Perhaps the most widely utilized endodontic irrigant is sodium hypochlorite, employed in varying dilutions. Since the results of this study reveal that when used as an endodontic irrigant, 5.25 percent sodium hypochlorite is a highly effective antibacterial agent against B. melaninogenicus, the vast majority of practitioners are probably eliminating this endodontic pathogen with their current treatment procedures. It would seem that as long as a 5.25 percent sodium hypochlorite solution is used as the root canal irrigant, and the solution is kept in contact with the canal and replenished often during the procedure, then the dentist can be relatively confident that the B. melaninogenicus

population has been killed or at least reduced to a level whereby the bodily defense mechanisms can complete the elimination process. If the practitioner does not routinely use sodium hypochlorite then it would seem prudent for him to do so, or at least consider the use of this solution whenever symptoms indicating the possibility of B. melaninogenicus are encountered.

Advantages of combined irrigation

Weine (102) suggests the use of Gly-Oxide in combination with sodium hypochlorite as root canal irrigants. He claims that the resultant liberation of oxygen will destroy strictly anaerobic microorganisms. Comparing the results displayed in Tables 6 and 8, the addition of Gly-Oxide to the irrigation procedure does not provide any germicidal advantage over sodium hypochlorite used alone, at least against B. melaninogenicus and P. anaerobius. Although the liberation of and exposure to oxygen may have some detrimental effect on anaerobic bacteria, contact of B. melaninogenicus and P. anaerobius with Clorox alone is sufficient to kill the bacteria. Combined irrigation with Gly-Oxide and sodium hypochlorite when used against anaerobic bacteria probably represents an overkill situation.

The antibacterial advantage attributed to effervescence may not be of clinical importance. However, two other

advantages for the use of an oxygenating agent in conjunction with sodium hypochlorite have been advanced. Marshall, Massler, and Dute (65) reported that this combination of irrigants significantly increased the permeability of dentinal tubules. These investigators considered this to be advantageous because it would allow increased penetration of the tubules by an intracanal medicament. Even more important are the findings of DeRenzis (103,104), who was able to demonstrate the deactivation of bacterial endotoxin by molecular oxygen and hydrogen peroxide. It may be that the use of combined irrigation is effective in deactivating the endotoxin released by B. melaninogenicus, thus limiting its pathogenicity. The clinical significance of these potential advantages is unknown. Further investigation of these possible advantages is recommended.

SUMMARY

A laboratory study was undertaken to appraise the bactericidal competency of various endodontic irrigants when used against the pulpal pathogen B. melaninogenicus. The effects of saline, Clorox, Gly-Oxide, and a combination of Clorox and Gly-Oxide were evaluated. P. anaerobius was included to simulate the clinical situation.

Tube dilution studies showed full-strength Clorox and full-strength Gly-Oxide to be powerful antibacterial agents against the test organisms. Dilution of the solutions diminished their bactericidal qualities, although Clorox diluted 1:15,000 rapidly killed B. melaninogenicus.

Experiments mimicking irrigation procedures in extracted teeth demonstrated that full-strength Clorox, and Clorox alternated with Gly-Oxide, had a 100 percent sterilizing effect on canals inoculated with B. melaninogenicus and P. anaerobius. Full-strength Gly-Oxide had a variable effect, while saline was totally ineffective at disinfecting the extracted teeth.

CONCLUSIONS

The following conclusions were drawn after completing this investigation:

1. Saline irrigation and exposure to atmospheric oxygen are totally ineffectual against B. melaninogenicus in the root canal.
2. Irrigation with 5.25 percent sodium hypochlorite is a safe and effective method of eliminating B. melaninogenicus from the pulp space. No change in the currently accepted irrigation regimen is required.
3. Combined irrigation alternating Gly-Oxide and sodium hypochlorite is no more effective at killing B. melaninogenicus than is sodium hypochlorite alone.
4. Further studies should consider the effect of combined irrigation on bacterial endotoxin.

REFERENCES

1. Miller, W.D.: An introduction to the study of the bacteriopathology of the dental pulp. Dental Cosmos, 36:505, 1894.

2. Rhein, M.L., Krasnow, F., and Gies, W.J.: A prolonged study of the electrolytic treatment of dental focal infection: a preliminary report. Dental Cosmos, 68:971, 1926.

3. Oliet, S.: Evaluation of culturing in endodontic therapy: a preliminary clinical report. Oral Surg., 15:727, 1962.

4. Zeldow, B.J., and Ingle, J.I.: Correlation of the positive culture to the prognosis of endodontically treated teeth: a clinical study. J. Am. Dent. Assoc., 66:23, 1963.

5. Bender, I.B., Seltzer, S., and Turkenkopf, S.: To culture or not to culture?. Oral Surg., 18:527, 1964.

6. Buchbinder, M.: A statistical comparison of cultured and noncultured root canal cases. J. Dent. Res., 20:93, 1941.

7. Kakehashi, S., Stanley, H.R., and Fitzgerald, R.J.: The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg., 20:340, 1965.

8. Torneck, C.: The role of microorganisms in endodontic disease. Alpha Omegan, 180, December, 1969.

9. Bartels, H.S., Naidorf, I., and Blechman, H.: A study of some factors associated with endodontic "flare-ups". Oral Surg., 25:255, 1968.

10. Sundqvist, G.: Bacteriologic studies of necrotic dental pulps. Umea Univ. Odontological Dissertations No. 7. Umea Sweden, 1976.

11. Griffee, M.B., Patterson, S.S., Miller, C.H., Kafrawy, A.H., and Newton, C.W.: The relationship of *Bacteroides melaninogenicus* to symptoms associated with pulpal necrosis. Oral Surg., 50:457, 1980.

12. Cran, J.H.: Study of the pathology and bacteriology of the pulpless tooth and its bearing on treatment. Aust. Dent. J., 1:161, 1956.

13. MacDonald, J.B., Hare, G.C., and Wood, A.W.S.: The bacteriologic status of the pulp chambers in intact teeth found to be non-vital following trauma. Oral Surg., 10:318, 1957.

14. Brown, L.R., and Rudolph, C.E.: Isolation and identification of microorganisms from exposed canals of pulp involved teeth, Oral Surg., 10:1094, 1957.

15. Leavitt, J.M., Naidorf, I., and Shugaevsky, P.: The bacterial flora of root canals as disclosed by a culture medium for endodontics. Oral Surg., 11:302, 1958.

16. Winkler, K.C., and van Amerongen, J.: Bacteriologic results of 4,000 root canal cultures. Oral Surg., 12:857, 1959.

17. Crawford, J.J., and Shankle, R.J.: Application of newer methods to study the importance of root canal and oral microbiota in endodontics. Oral Surg., 14:1109, 1961.

18. Engstrom, B., and Frostell, G.: Bacteriological studies of the non-vital pulp in cases with intact pulp cavities. Acta. Odont. Scand., 19:23, 1961.

19. Möller, A.J.R.: Microbial examination of root canals and periapical tissues of human teeth. Akademiforlegat, Goteborg, Sweden, 1966.

20. Leavitt, J.M., Naidorf, I., and Shugaevsky, P.: The undetected anaerobe in endodontics. New York Dent. J., 25:377, 1955.

21. Finegold, S.M.: Anaerobic Bacteria in Human Disease, New York, Academic Press, 1977.

22. Fulghum, R.S., Wiggins, C.B., and Mullaney, T.R.: Pilot study for detecting obligate anaerobic bacterium in necrotic dental pulps. J. Dent. Res., 52:637, (abst.) 1973.

23. Bergenholtz, G.: Microorganisms from necrotic pulps of traumatized teeth. Odont. Revy., 25:347, 1974.

24. Kantz, W.E., and Henry, C.A.: Isolation and classification of anaerobic bacteria from intact pulp chambers of non-vital teeth in man. Arch. Oral Bio., 19:91, 1974.

25. Wittgow, W.C., and Sabiston, C.B.: Microorganisms from pulpal chambers of intact teeth with necrotic pulps. J. Endod., 1:168, 1975.

26. Keudell, K., Conte, M., Fujimoto, L., Ernest, M., and Berry, H.G.: Microorganisms isolated from pulp chambers. J. Endod., 2:146, 1976.

27. Zielke, D.R., Heggers, J.P., and Harrison, J.W.: A statistical analysis of anaerobic versus aerobic culturing in endodontic therapy. Oral Surg., 42:830, 1976.

28. Goodman, A.D.: Isolation of anaerobic bacteria from the root canal systems of necrotic teeth using a transport solution. Oral Surg., 42:766, 1977.

29. Oliver, W.W., and Wherry, W.R.: Notes on some bacterial parasites of human mucous membranes. J. Infect. Dis., 28:341, 1921.

30. Schwabacher, H., Lucas, P.R., and Rimington, C.: Bacterium melaninogenicum - a misnomer. J. Gen. Microbiol., 1:109, 1947.

31. Williams, R.A.D., Bowden, G.H., Hardie, J.M., and Shah, H.: Biochemical properties of Bacteroides melaninogenicus subspecies. Int. J. Syst. Bacteriol., 25:298, 1975.

32. Finegold, S.M., and Barnes, E.M.: Report of the ICSB Taxonomic Subcommittee on gram-negative anaerobic rods. Int. J. Syst. Bacteriol., 27:388, 1977.

33. Coykendall, A.L., Kaczmarek, F.S., and Slots, J.: Genetic heterogeneity in Bacteroides asaccharolyticus and proposal of Bacteroides gingivalis. Int. J. Syst. Bacteriol., 30:559, 1980.

34. Burdon, K.L.: Bacterium melaninogenicum from normal and pathologic tissues. J. Infect. Dis., 42:161, 1928.

35. Socransky, S.S., and Gibbons, R.J.: Required role of Bacteroides melaninogenicus in mixed infections. J. Infect. Dis., 115:247, 1965.

36. Lev, M.: Apparent requirements for vitamin K of rumen strains of Fusiform nigrescens. Nature, 181:203, 1958.

37. Gibbons, R.J., and MacDonald, J.B.: Requirements of hemin and vitamin K by certain strains of Bacteroides melaninogenicus. J. Dent. Res., 39:655, 1960.

38. MacDonald, J.B., Socransky, S.S., and Gibbons, R.J.: Aspects of the pathogenesis of mixed anaerobic infections of mucous membranes. J. Dent. Res., 42:529, 1963.

39. Keudell, K., and Conte, M.: Enzymes of microbial isolates from infected pulp chambers. J. Endod., 2:217, 1976.

40. MacDonald, J.B., and Gibbons, R.J.: Hydrolysis of collagen by cultures of Bacteroides melaninogenicus. J. Dent. Res., 39:655, 1960 (abstract).

41. Sawyer, S.J., MacDonald, J.B., and Gibbons, R.J.: Biochemical characteristics of Bacteroides melaninogenicus. Arch. Oral Biol., 7:685, 1962.

42. Schein, B., and Schilder, H.: Endotoxin content of endodontically involved teeth. J. Endod., 1:19, 1975.

43. Tally, F.P., Goldin, B.R., Jacobus, N.V., and Gorbach, S.L.: Superoxide dismutase in anaerobic bacteria of clinical significance. Infect. Immun., 16:20, 1977.

44. Rolfe, R.D., Hentges, D.J., Campbell, B.J., and Barrett, J.T.: Factors related to the oxygen tolerance of anaerobic bacteria. Appl. Environ. Microbiol., 36:306, 1978.

45. Gregory, E.M., Moore, E.C., and Holdeman, L.V.: Superoxide dismutase in anaerobes: survey. Appl. Environ. Microbiol., 35:988, 1978.

46. Wilkins, T.D., Wagner, D.L., Veltri, B.J., and Gregory, E.M.: Factors affecting the production of catalase by Bacteriodes. J. Clin. Microfiol., 8:553, 1978.

47. Loesch, W.J.: Oxygen sensitivity of various anaerobic bacteria. Appl. Microbiol., 18:723, 1969.

48. Prader, F.: Schwiez. Monatsschr. f. Zhk., 57:383, 1947. in Grossman, L.I.: Endodontic Practice, 10th ed., Philadelphia, Lea and Febiger, 1981, pg. 237.

49. Blechman, H., Cohen, M: Use of aqueous urea solution in the field of endodontics: a preliminary report. J. Dent. Res., 30:503, 1951.

50. Walker, A.: A definite and dependable therapy for pulpless teeth. J. Amer. Dent. Assoc., 23:1418, 1936.

51. Coolidge, E.D., and Kesel, R.G.: Textbook of Endodontology, 2nd ed., Philadelphia, Lea and Febinger, 1956, pg. 200.

52. Stewart, GG., Cobe, H.M., and Rappaport, H.: A study of a new medicament in the chemomechanical preparation of infected root canals. J. Amer. Dent. Assoc., 63:33, 1961.

53. Nichols, E.: The efficacy of cleansing of the root canal. Brit. Dent. J., 112:167, 1962.

54. Grahnen, H., and Krasse, B.: The effect of instrumentation and flushing of non-vital teeth in endodontic therapy. Odont. Revy., 14:167, 1963.

55. Grossman, L.I.: Irrigation of root canals. J. Amer. Dent. Assoc., 30:1915, 1943.

56. Callahan, J.R.: Sulfuric acid for opening root canals. Dent. Cosmos, 36:957, 1894.

57. Kirk, E.C.: Note to the editor. Dent. Cosmos, 35:162, 1893.

58. Wilkinson, F.C.: Root canal treatment: report of some investigations dealing with the elimination of necrotic tissue from the root canal. Brit. Dent. J., 50:1, 1929.

59. Baker, N.S., Eleazer, P.D., Averbach, R.E., and Seltzer, S.S.: Scanning electron microscope study of the efficacy of various irrigating solutions. J. Endod., 1:127, 1975.

60. McComb, D., and Smith, D.C.: A preliminary scanning electron microscopic study of root canals after endodontic procedures. J. Endod., 1:238, 1975.

61. Vande Visse, J.E., and Brilliant, J.D.: Effect of irrigation on the production of extruded material at the root apex. J. Endod., 1:243, 1975.

62. Ram, Z.: Effectiveness of root canal irrigation. Oral Surg., 44:306, 1977.

63. Svec, T.A., and Harrison, J.W.: Chemomechanical removal of pulpal and dentinal debris with sodium hypochlorite and hydrogen peroxide vs. normal saline. J. Endod., 3:49, 1977.

64. Svec, T.A., and Harrison, J.W.: The effect of effervescence on debridement of the apical regions of root canals in single rooted teeth. J. Endod., 7:335, 1981.

65. Marshall, F.J., Massler, M., and Dute, H.L.: Effects of endodontic treatments on permeability of root dentin. Oral Surg., 13:208, 1960.

66. Harrison, J.W., and Hand, R.E.: The effect of dilution and organic matter on the antibacterial property of 5.25 percent sodium hypochlorite. J. Endod., 7:128, 1981.

67. Brown, J.I., and Doran, J.E.: An *in vitro* evaluation of the particle floatation capability of various irrigating solutions. J. Calif. Dent. Assoc., 3:60, 1975.

68. Dakin, H.D.: On the use of certain antiseptic substances in the treatment of infected wounds. Brit. Med. J., 2:318, 1915.

69. Austin, J.H., and Taylor, H.D.: Behavior of hypochlorite solution in contact with necrotic and normal tissue *in vivo*. J. Exp. Med., 27:627, 1918.

70. Taylor, H.D., and Austin, J.H.: The solvent action of antiseptics on necrotic tissue. J. Exp. Med., 27:155, 1918.

71. Lewis, P.: Sodium hypochlorite root canal therapy. J. Fla. Dent. Soc., 24:10, 1954.

72. Grossman, L.I., and Meiman, B.W.: Solution of pulp tissue by chemical agents. J. Amer. Dent. Assoc., 28:223, 1941.

73. Senia, E.S., Marshall, F.J., and Rosen, S.: The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. Oral Surg., 31:96, 1971.

74. Masterton, J.B.: Chemical debridement in the treatment of infected pulpless teeth and chronic periapical abscess. Dent. Pract., 15:162, 1965.

75. Bence, R., and others: A microbiologic evaluation of endodontic instrumentation to pulpless teeth. Oral Surg., 35:676, 1973.

76. Costigan, S.M.: Comparison of germicidal efficiency of hypochlorite of high and low alkalinity. J. Bact., 34:1, 1937.

77. Cvek, M.: Antimicrobial effect of root canal debridement: a clinical and microbiologic study. Odont. Revy., 27:1, 1976.

78. Shih, M., Marshall, F.J., and Rosen, S.: Bactericidal efficiency of sodium hypochlorite as an endodontic irrigant. Oral Surg., 29:613, 1970.

79. Raphael, D., Wong, T.A., Moodnik, R., and Borden, B.G.: The effect of temperature on the bactericidal efficiency of sodium hypochlorite. J. Endod., 7:330, 1981.

80. Spangberg, L.: Cellular reaction to intracanal medicaments. In Grossman, L.I. (ed.): Trans. Fifth Int. Conf. Endod., Philadelphia, Univ. of Pennsylvania, 1973, pp. 108-123.

81. Trepagnier, C.M., Madden, R.M., and Lazzari, E.P.: Quantitative study of sodium hypochlorite as an *in vitro* endodontic irrigant. J. Endod., 3:194, 1977.

82. Harrison, J.W., Svec, T.A., and Baumgartner, J.C.: Analysis of clinical toxicity of endodontic irrigants. J. Endod., 4:6, 1978.

83. Hand, R.E., Smith, M.L., and Harrison, J.W.: Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J. Endod., 4:60, 1978.

84. Babior, B.M.: Oxygen-dependent microbial killing by phagocytes. New Eng. J. Med., 298:659, 1978.

85. Cobe, H.M.: Investigations of a new dental chemotherapeutic agent in the presence of blood. Oral Surg., 13:678, 1960.

86. Robinson, W.: Use of urea to stimulate healing in chronic purulent wounds. Am. J. Surg., 33:192, 1936.

87. Brown, E.A., Abramson, H.A., Gorin, M.H., Kauffman, H.O., and Shanley, E.S.: The stability of solutions of urea peroxide in glycerol, isopropanol, and ethylene glycol. J. Am. Pharm. Assoc., 35:304, 1946.

88. Brown, E.A., Skiffington, R.E., and Krabek, W.B.: Glycerite of hydrogen peroxide: a comparison of its bacteriotoxic effect on Clostridia with that of other antiseptics. Am. J. Surg., 77:630, 1949.

89. Thurmon, F., and Brown, E.A.: Studies of the effects of glycerite of hydrogen peroxide upon infections of the skin and mucous membranes. J. Invest. Dermatol., 8:11, 1947.

90. Brown, E.H., Krabek, W., and Skiffington, R.E.: Glycerite of hydrogen peroxide: comparison of its bacteriostatic action with that of mercurial solutions. J. Bacteriol., 53:793, 1947.

91. Slantez, L.W., and Brown, E.A.: The dimunition of the numbers of oral organisms following the use of carbamide peroxide in glycerol. J. Bacteriol., 51:126, 1946.

92. Slantez, L.W., and Brown, E.A.: Studies of the effect of glycerite of hydrogen peroxide upon the numbers of oral microorganisms. J. Dent. Res., 25:223, 1946.

93. Brown, E.A., Krabek, W., and Skiffington, R.E.: A new antiseptic solution for topical application. N. Eng. Med. J., 234:468, 1946.

94. Brown, E.A.: Studies on tissue tolerance to a new glycerol peroxide antiseptic solution. Ann. Allerg., 4:33, 1946.

95. Gillan, J.W.: A short-term study of the effects of Gly-Oxide on periapical tissues in the rhesus monkey. Thesis, Loyola Univ. of Chicago, 1979.

96. Cobe, H.M., Cohen, D.W., and Hattler, A.D.: Urea peroxide in glycerine: a preliminary study of its value as an adjunct to periodontal therapy. Penn. Dent. J., 25:12, 1959.

97. Epstein, S.: Clinical comparison of thickened and unthickened urea-peroxide glycerine products. Oral Surg., 32:886, 1971.

98. Wagner, H.P.: The use of a chemotherapeutic agent as an aid in the healing of post-operative extraction wounds. D.C. Dent. Soc. J., 37:12, 1962.

99. Weine, F.S.: Endodontic Therapy, 2nd ed. St. Louis, C.V. Mosby Co., 1976, pp. 215-216.

100. Marshall, F.J., and Savoie, F.L.: Efficiency of endodontic culturing using wet and dry paper points. Oral Surg., 23:806, 1967.

101. Grossman, L.I.: Endodontic Practice, 10th ed. Philadelphia, Lea and Febiger, 1981, pg. 250.

102. Weine, F.S.: Endodontic Therapy, 2nd ed. St. Louis, C.V. Mosby Co., 1976, pg. 229.

103. De Renzis, F.A.: Endotoxin-inactivating potency of hydrogen peroxide: effect on cell growth. J. Dent. Res., 60:933, 1981.

104. De Renzis, F.A.: Endotoxin-inactivating potency of molecular oxygen: effect on cell growth. J. Dent. Res., 59:1521, 1980.

APPROVAL SHEET

The thesis submitted by David B. Foley has been read and approved by the following committee:

Dr. James C. Hagen, Director
Assistant Professor, Microbiology, Loyola

Dr. Franklin S. Weine
Professor, Endodontics, Loyola

Dr. Scott B. Shellhammer
Associate Professor, Endodontics, Loyola

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

3/1/82
Date

James C. Hagen, Ph.D.
Director's Signature